A POTENCY TEST OF TUBERCULIN

KIZASHI YOSHINAGA

Ishigami Memorial Institute, Hamadera, Osaka, Japan.

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INTRODUCTORY REMARKS

In this paper we will deal chiefly with the potency test of a tuberculin on guinea pigs. To discuss the test, we will begin with an example showing how to calculate the potency of a tuberculin. And the discussions of finer points about the principles on which our method is based will be put off till the later sections.

It is beyond the scope of this paper to describe the details of factorial designs. The reader is advised to refer to the textbook of Masuyama's "Jikken Keikakuho Gaiyo," (An Outline of Experimental Designs). As for the theory of correlation, there are so many books on statistics that we need not to quote any of them here.

Guinea pigs are sensitized by dead tubercule bacilli suspended in liquid paraffin. Before the potency test the animals are tested for their grades of sentitization by injecting 0.1 ml of 1:2000 dilution of OT intracutaneously. Among those showing a reaction of induration of diameter 10 mm or more 24 hours later we pick up 3 or 4 animals having about the same size of reaction.

In the first place, we will state a three-point-measurement which is now in common use in our research; and in the second a two-point-measurement which we used in former days.
CHAPTER 1. THE METHOD OF POTENCY TEST
WITH TWO EXAMPLES

§ 1.1 AN EXAMPLE OF THREE-POINT-MEASUREMENT. A example
of testing the potency of a tuberculin M2 against the standard M1 by means
of the three-point-measurement is given as follows:

1. Both tuberculins, M1 and M2 are diluted in three grades; V1 is not
diluted, V2 is diluted 2 times with buffer solution, V3 is diluted 4 times.
M1V1 means V1 of M1, M1V2 means V2 of M1; M2V1 means V1 of M2,
and so on.

2. To make injections, the hair of the guinea pig is clipped off at 18
points, 9 on the right, 9 on the left side.

3. The injection is made intracutaneously in a volume of 0.1 ml.

4. After 24 hours, the measurement is made on induration and redness.
For the sake of simplicity we will state here the readings on induration only.

5. Q1, Q2 and Q3 are three pairs of lines parallel to the backbone of
the animal. Q1 lies near the backbone, Q3 near the abdomen, and Q2 inter-
mediate.

See table 1.1.1.

<table>
<thead>
<tr>
<th>Guinea pig</th>
<th>M1V2 11×10</th>
<th>M1V3 9×8</th>
<th>M1V1 14×14</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 Head</td>
<td>Q3</td>
<td>Q1</td>
<td>Q2</td>
</tr>
<tr>
<td>Q1</td>
<td>M2V1 15×13</td>
<td>M2V2 9×10</td>
<td>M2V3 9×9</td>
</tr>
<tr>
<td>Q2</td>
<td>M1V3 10×10</td>
<td>M1V1 12×12</td>
<td>M1V2 10×10</td>
</tr>
<tr>
<td>Q3</td>
<td>M2V2 10×11</td>
<td>M2V3 10×10</td>
<td>M2V1 12×12</td>
</tr>
<tr>
<td>Q4</td>
<td>M1V1 12×10</td>
<td>M1V2 10×9</td>
<td>M1V3 9×8</td>
</tr>
<tr>
<td>Q5</td>
<td>M2V2 9×8</td>
<td>M2V3 7×8</td>
<td>M2V1 10×9</td>
</tr>
<tr>
<td>Q6</td>
<td>M1V3 7×7</td>
<td>M1V1 11×11</td>
<td>M1V2 9×9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N2 Head</th>
<th>M2V3 6×6</th>
<th>M2V1 10×11</th>
<th>M2V2 8×8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>M2V2 10×10</td>
<td>M1V3 8×8</td>
<td>M1V1 12×12</td>
</tr>
<tr>
<td>Q2</td>
<td>M2V1 13×12</td>
<td>M2V2 7×8</td>
<td>M2V3 6×7</td>
</tr>
<tr>
<td>Q3</td>
<td>M1V3 9×8</td>
<td>M1V1 12×11</td>
<td>M1V2 11×9</td>
</tr>
<tr>
<td>Q4</td>
<td>M2V1 12×10</td>
<td>M2V2 9×9</td>
<td>M2V3 8×8</td>
</tr>
<tr>
<td>Q5</td>
<td>M1V2 9×9</td>
<td>M1V3 7×8</td>
<td>M1V1 10×10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N3 Head</th>
<th>M2V2 10×9</th>
<th>M2V3 8×9</th>
<th>M2V1 10×10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>M2V1 12×11</td>
<td>M1V2 11×10</td>
<td>M1V3 8×9</td>
</tr>
<tr>
<td>Q2</td>
<td>M2V3 9×9</td>
<td>M2V1 12×11</td>
<td>M2V2 11×10</td>
</tr>
</tbody>
</table>

The arithmetical means of long and short diameters are made and rear-
ranged in the following way (table 1.1.2):
To facilitate the computations we make the “codings” of the figures given in table 1.1.2. Let these figures be denoted by $Y$, and making $Y = 10Y - 100$, we have table 1.1.3.

**Table 1.1.3. Made from table 1.1.2 by coding the numbers.**

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th></th>
<th>M2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V2</td>
<td>V3</td>
<td>V1</td>
</tr>
<tr>
<td>N1</td>
<td>Q1</td>
<td>12.5</td>
<td>10.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Q2</td>
<td>12</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Q3</td>
<td>14</td>
<td>10.5</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Q1</td>
<td>11</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>N2</td>
<td>Q2</td>
<td>12</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Q3</td>
<td>11</td>
<td>9.5</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Q1</td>
<td>10</td>
<td>9</td>
<td>7.5</td>
</tr>
<tr>
<td>N3</td>
<td>Q2</td>
<td>11.5</td>
<td>10.5</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Q3</td>
<td>11.5</td>
<td>10</td>
<td>8.5</td>
</tr>
</tbody>
</table>

$\Sigma y = 155 - 10 - 145 = 130 - 65 - 155$

$\Sigma y^2 = 3675 300 3225 3400 1475 4275$

Make table 1.1.4 from the last-but-one line of table 1.1.3.

**Table 1.1.4 Made from the $\Sigma y$-line of table 1.1.3.**

<table>
<thead>
<tr>
<th>V1</th>
<th>V2</th>
<th>V3</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>155</td>
<td>-10</td>
</tr>
<tr>
<td>M2</td>
<td>130</td>
<td>-65</td>
</tr>
</tbody>
</table>

$C = (-90)^2 / 54 = 150$. This $C$ is called correction term. 54 is the number of items of table 1.1.3.
From the last line of table 1.1.3 we have
\[ S_T = 3675 + 300 + 3225 + 3400 + 1475 + 4275 - 150 = 16200 \]
\[ s_y^2 = S_T/54 = 300 \]

Then we make a sum of products \("p\) of \(x\) and \(y\), \(x\) being the logarithms of dilutions coded by taking \(V_2\) as the origin. Combining the last line of table 1.1.4 and the \(x\), we have the following table 1.1.5.

**Table 1.1.5 To compute \("p\).**

<table>
<thead>
<tr>
<th></th>
<th>(V_1)</th>
<th>(V_2)</th>
<th>(V_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(y)</td>
<td>285</td>
<td>-75</td>
<td>-300</td>
</tr>
<tr>
<td>(x)</td>
<td>-1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>-285</td>
<td>0</td>
<td>-300</td>
</tr>
</tbody>
</table>

\[ p = \frac{-585}{54} - \bar{x} \bar{y} = -10.83 - 0 \times -10.83 = -10.83 \]

And

\[ s_x^2 = 2/3 = 0.6667 \]
\[ a = p/s_x^2 = -10.83/0.6667 = -16.25 \]
\[ \beta = p/s_y^2 = -10.83/300 = -0.03610 \]
\[ r^2 = a\beta = 0.5866 \]
\[ r = -0.7659 \]

The reader is advised to refer to a textbook of statistics to learn how to calculate a correlation coefficient. The correlation is calculated to see if the experiment is suitable for the estimation of potency. If the correlation coefficient is very small, say 0.4 or under in its absolute value, the potency estimated will be very close to unity, while it should be far from 1.0 in reality.

The regression coefficient \(\beta\) is only of use for the calculation of potency.

Then we make an average of \(M_2-M_1\), that is

\[ d = \frac{(M_2-M_1)}{27} = -90/27 = -3.333 \]
\[ \log 0_\beta = 0.301 \times d \times |\beta| = -0.301 \times 3.333 \times 0.03610 = -0.03622 = 1.96378 \]
\[ 0_\beta = 0.9200 \]

This \(0_\beta\) is an estimate of the potency of \(M_2\) against \(M_1\). 0.301 is the logarithm of 2 which is the multiple of diluting from \(V_1\) to \(V_2\), and from \(V_2\) to \(V_3\).

The estimate may not be correct if the experiment is not adequately carried out, for instance if the correlation coefficient is too small. A confirmatory test is required to see if \(M_2\) is equivalent to \(M_1\) which is diluted \(1/0_\beta\) times. \(M_1\) is diluted \(1/0.92 = 1.09\) times. Then \(M_2\) must be of equal potency to the diluted \(M_1\), if the estimate at 0.92 is correct. The procedure is the same as stated above. If the estimate is larger than 1.0, then \(M_2\) must be diluted and compared with \(M_1\).

\[ \S 1.1.(a) \text{ ANOTHER METHOD OF CALCULATION.} \]

The reader having some skill in algebra will easily prove the equation:

\[ \log 0_\beta = 2 \times 0.301(V_1-V_3) (M_2-M_1)/nS_T, \]
where n is the number of items. This equation holds good for three-point-measurement only. Since $2 \times 0.301/n$ is constant in an experiment, we will omit this fraction; and the remainder $(M_2-M_1)(V_1-V_3)/S_T$ is a coded number of log$	heta$. 

Now see table 1.1.3 once more. Following the principle stated in section 1.1, we are able to compute the log$	heta$ on each line of NQ of table 1.1.3. And we shall have nine values of potencies. The computations are made in the following way:— See table 1.1.(a).A and 1.1.(a).B On table 1.1.(a),A we compute $(M_2-M_1)(V_1-V_3)$.

### Table 1.1.(a).A Computation of $(M_2-M_1)(V_1-V_3)$

<table>
<thead>
<tr>
<th>M1</th>
<th>M2</th>
<th>M2-M1</th>
<th>V1</th>
<th>V3</th>
<th>V1-V3</th>
<th>$(M_2-M_1)(V_1-V_3)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>25</td>
<td>-5</td>
<td>65</td>
<td>-10</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>25</td>
<td>5</td>
<td>40</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>25</td>
<td>-5</td>
<td>60</td>
<td>-15</td>
<td>75</td>
</tr>
<tr>
<td>1</td>
<td>-30</td>
<td>-55</td>
<td>-25</td>
<td>15</td>
<td>-70</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>-45</td>
<td>45</td>
<td>15</td>
<td>-45</td>
</tr>
<tr>
<td>3</td>
<td>-20</td>
<td>-35</td>
<td>-15</td>
<td>35</td>
<td>-60</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>-35</td>
<td>-20</td>
<td>15</td>
<td>0</td>
<td>-40</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>-20</td>
<td>-25</td>
<td>25</td>
<td>-35</td>
<td>60</td>
</tr>
</tbody>
</table>

On table 1.1.(a).B we compute $s_y^2$ in each row, the correction term being made for each. Then we make $(M_2-M_1)(V_1-V_3)/s_y^2$, a coded value of log$	heta$, which we will denote by $\theta$. Averaging the nine $\theta$'s, we have $\theta_0$. To compute the standard error of $\theta_0$, we make $\theta-\theta_0$, and their squares.

### Table 1.1.(a).B To compute $s_y^2$ and make $(M_2-M_1)(V_1-V_3)/s_y^2$ and find the standard error.

<table>
<thead>
<tr>
<th>Sum of squares of entries</th>
<th>Sum</th>
<th>Correction term</th>
<th>$s_y^2$</th>
<th>$(M_2-M_1)(V_1-V_3)$</th>
<th>$\theta-\theta_0$</th>
<th>$(\theta-\theta_0)^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2375</td>
<td>55</td>
<td>504</td>
<td>1871</td>
<td>-0.200</td>
<td>0.147</td>
</tr>
<tr>
<td>2</td>
<td>825</td>
<td>45</td>
<td>338</td>
<td>487</td>
<td>0.411</td>
<td>0.758</td>
</tr>
<tr>
<td>3</td>
<td>2275</td>
<td>55</td>
<td>504</td>
<td>1771</td>
<td>-0.212</td>
<td>0.135</td>
</tr>
<tr>
<td>2</td>
<td>3125</td>
<td>-85</td>
<td>1204</td>
<td>1921</td>
<td>-1.106</td>
<td>-0.759</td>
</tr>
<tr>
<td>2</td>
<td>1675</td>
<td>-45</td>
<td>338</td>
<td>1337</td>
<td>-2.019</td>
<td>-1.672</td>
</tr>
<tr>
<td>3</td>
<td>3225</td>
<td>-55</td>
<td>504</td>
<td>2721</td>
<td>-0.524</td>
<td>-0.177</td>
</tr>
<tr>
<td>1</td>
<td>975</td>
<td>-55</td>
<td>504</td>
<td>471</td>
<td>1.274</td>
<td>1.621</td>
</tr>
<tr>
<td>3</td>
<td>1075</td>
<td>-15</td>
<td>38</td>
<td>1037</td>
<td>-1.447</td>
<td>-1.100</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>10</td>
<td>17</td>
<td>783</td>
<td>0.702</td>
<td>1.049</td>
</tr>
</tbody>
</table>

$\theta_0 = -3.121/9 = -0.347$
By decoding the code number $\theta_0$, we shall have the potency as follows:
\[
\log\theta_0 = (-0.347)(2)(0.301)/6 = -0.0348 = 1.9652
\]
\[
\theta_0 = 0.9230
\]
This value 0.9230 is almost equal to 0.9200 obtained in preceding section. Exact accordance of both values can never be expected. It is not surprising that we should have some discrepancy between two values. Because, in section 1.1 M2–M1 is averaged beforehand and the regression coefficient is obtained by pooling $y$'s on the whole; and the potency, $\log\theta_0$, is obtained afterwards by multiplying $d$ by $|\beta|$. In this section we make an elemental $\log\theta_0$ on each line of NQ, and nine elements of potency are averaged in the last.

The operations are more cumbersome in this section. But we are rewarded by knowing the confidence limits of potency estimated. The standard error of $\theta_0$ will be obtained as follows:
\[
S\theta_{0}^2 = s^2 / n = \frac{s_2^2}{(n-1)n} = \frac{8.95543}{(8)(9)} = 0.1244
\]
\[
S\theta_{0} = 0.3527
\]
Referring to the table of "t" you will find the value 2.306 for degree of freedom $n-1=9-1=8$ at the level of probability 0.05. Now we have
\[
t_{0.05}s\theta_{0} = (2.306)(0.3527) = 0.813
\]
The upper fiducial limit of $\theta_0$ is:
\[
L_1 = \theta_0 + t_{0.05}s\theta_{0} = -0.347 + 0.813 = 0.466
\]
The lower fiducial limit of $\theta_0$ is:
\[
L_2 = \theta_0 - t_{0.05}s\theta_{0} = -0.347 - 0.813 = -1.160
\]
By decoding we have the upper limit of $\log\theta_0$:
\[
\log\theta_{01} = 0.466 \times \frac{0.301 \times 2}{6} = 0.0468
\]
\[
\theta_{01} = 1.114
\]
Similarly we have the lower limit of $\log\theta_0$:
\[
\log\theta_{02} = (-1.160)(0.301)(2)/6 = -0.1164 = 1.8836
\]
\[
\theta_{02} = 0.7649
\]
The population mean of elemental potencies lies between 0.76 and 1.1. This shows that the potency, although we have estimated it at 0.92, is not distinguishable from 1.0 as yet. So we are not successful to prove any significant difference between M1 and M2 in this experiment.

§ 1.2 AN EXAMPLE OF TWO-POINT-MEASUREMENT. Two classes of dilutions of the test and the standard tuberculin are injected intracutaneously in a volume of 0.1 ml. In table 1.2.1 M1 means the standard, M2 the test. V1 means 2000 times dilution, V2 4000 times. On the top of the table we see $10 \times 11$, which means an induration of 10 mm length by 11 mm width. 10.5 is the arithmetical mean of 10 and 11. 3.24 is the square root of the arithmetical mean.
Table 1.2.1 An example of two-point-measurement.

<table>
<thead>
<tr>
<th>Guinea pig</th>
<th>Right</th>
<th>M2V2</th>
<th>M1V1</th>
<th>M2V1</th>
<th>M1V2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head</td>
<td>10×11</td>
<td>14×17</td>
<td>16×16</td>
<td>9×10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.5</td>
<td>15.5</td>
<td>16.0</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.24</td>
<td>3.94</td>
<td>4.0</td>
<td>3.08</td>
</tr>
<tr>
<td>N2</td>
<td>Left</td>
<td>10×11</td>
<td>16×16</td>
<td>16×17</td>
<td>10×10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.5</td>
<td>16.0</td>
<td>16.5</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.24</td>
<td>4.00</td>
<td>4.06</td>
<td>3.16</td>
</tr>
<tr>
<td>N3</td>
<td>M2V1</td>
<td>M1V2</td>
<td>M2V2</td>
<td>M1V1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12×13</td>
<td>8×10</td>
<td>8×10</td>
<td>14×10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>9.0</td>
<td>9.0</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.54</td>
<td>3.00</td>
<td>3.00</td>
<td>3.46</td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>M1V1</td>
<td>M2V2</td>
<td>M1V2</td>
<td>M2V1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11×13</td>
<td>8×9</td>
<td>10×11</td>
<td>10×13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>8.5</td>
<td>10.5</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.46</td>
<td>2.92</td>
<td>3.24</td>
<td>3.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2V2</td>
<td>M1V1</td>
<td>M1V2</td>
<td>M2V1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8×9</td>
<td>12×13</td>
<td>9×9</td>
<td>13×10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>12.5</td>
<td>9.0</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.92</td>
<td>3.54</td>
<td>3.00</td>
<td>3.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2V1</td>
<td>M1V2</td>
<td>M1V1</td>
<td>M2V2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11×12</td>
<td>9×8</td>
<td>12×11</td>
<td>8×7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.5</td>
<td>8.5</td>
<td>11.5</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.39</td>
<td>2.92</td>
<td>3.39</td>
<td>2.74</td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>M1V2</td>
<td>M2V1</td>
<td>M2V2</td>
<td>M1V1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9×10</td>
<td>13×14</td>
<td>9×10</td>
<td>18×15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>13.5</td>
<td>9.5</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.08</td>
<td>3.67</td>
<td>3.08</td>
<td>4.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1V1</td>
<td>M2V2</td>
<td>M2V1</td>
<td>M1V2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12×13</td>
<td>9×10</td>
<td>15×15</td>
<td>15×13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>9.5</td>
<td>15.0</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.54</td>
<td>3.08</td>
<td>3.87</td>
<td>3.74</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.2.1 is shown in its simplest form for the sake of simplicity. In practice, it is desirable—maybe indispensable to avoid a bias of sampling—to duplicate this form, alternating right and left sides. V1 and V2 may take other values, for instances, 500 and 2000, or 1000 and 2000 etc. This method is not the same as that of Umezawa’s (1), from which it differs in two principles; (A) the sites of injections, and (B) the calculation of figures.

(A) The sites of injections are modified from Umezawa’s new method in three points. (a) We have set up another plan against the local differences in the body of a guinea pig. The size of the reactions becomes gradually larger towards the tail, as it will be shown later. To counterbalance this inconvenience, the order of V1 and V2 is reversed in the same individual, i.e. (Head, V1, V2, V2, V1, Tail). These positions are again reversed in the second animal, i.e. (Head, V2, V1, V1, V2, Tail). (b) In the potency test, the differences of reactions between V1 and V2 are as equally important as that of the standard and the test tuberculin. In view of this point, V1 and V2 of one specimen are tested on the same “segment” of one individual, as shown on the third animal. On the fourth animal the sites of injections are reversed against the third. (c) Considering the difference, if any, between right and left sides, we made four injections of one specimen on zig-zag course between both sides. This is said about the first and second
animals. In the third and fourth animals one kind of dilutions takes a zig-zag course.

(B) THE CALCULATION OF FIGURES. The square roots of the arithmetical means of long and short diameters of indurations are extracted according to Masuyama-Umezawa's law. The figures are tabulated in table 1.2.2.

Table 1.2.2 The result rearranged.

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th></th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V2</td>
<td>V1</td>
</tr>
<tr>
<td>N1</td>
<td>3.94</td>
<td>3.24</td>
<td>4.00</td>
</tr>
<tr>
<td>Q1</td>
<td>4.06</td>
<td>3.08</td>
<td>4.00</td>
</tr>
<tr>
<td>Q2</td>
<td>3.46</td>
<td>3.00</td>
<td>3.54</td>
</tr>
<tr>
<td>N2</td>
<td>3.46</td>
<td>3.24</td>
<td>3.39</td>
</tr>
<tr>
<td>Q1</td>
<td>3.54</td>
<td>2.92</td>
<td>3.39</td>
</tr>
<tr>
<td>Q2</td>
<td>3.39</td>
<td>3.00</td>
<td>3.39</td>
</tr>
<tr>
<td>N3</td>
<td>3.54</td>
<td>3.08</td>
<td>3.67</td>
</tr>
<tr>
<td>Q1</td>
<td>4.06</td>
<td>3.74</td>
<td>3.87</td>
</tr>
<tr>
<td>Q2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>29.45</td>
<td>25.30</td>
<td>29.25</td>
</tr>
</tbody>
</table>

Notes:— Q1.....Fore half of the body, Q2 hind half.

To carry out analyses of factors (M, V, N and Q) we will deal with code numbers "y" instead of original figures "Y".

\[ y = 100 \frac{Y}{338} \]

338 is almost equal to \((100)(108.14)/32\), the arithmetical mean of 100 Y. But it is not necessary to calculate this mean every time. It is more convenient to use always 300. Because, it is easily subtracted, and the remaining figures are not so large as to disturb us when we deal with their squares. Besides, we will see later that the figure 300 is almost equal to the mean of figures measured by four factors, i.e. induration, redness, 24 hours' and 48 hours' reading.

Now, using 338, we have the following table 1.2.3.

Table 1.2.3 Working table made from code numbers of table 1.2.2.

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th></th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V2</td>
<td>V1</td>
</tr>
<tr>
<td>N1</td>
<td>56</td>
<td>-14</td>
<td>62</td>
</tr>
<tr>
<td>Q1</td>
<td>68</td>
<td>-30</td>
<td>62</td>
</tr>
<tr>
<td>Q2</td>
<td>8</td>
<td>-38</td>
<td>16</td>
</tr>
<tr>
<td>N2</td>
<td>8</td>
<td>-14</td>
<td>1</td>
</tr>
<tr>
<td>Q1</td>
<td>16</td>
<td>-46</td>
<td>1</td>
</tr>
<tr>
<td>Q2</td>
<td>1</td>
<td>-38</td>
<td>1</td>
</tr>
<tr>
<td>N3</td>
<td>16</td>
<td>-30</td>
<td>29</td>
</tr>
<tr>
<td>Q1</td>
<td>68</td>
<td>36</td>
<td>49</td>
</tr>
<tr>
<td>Q2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>241</td>
<td>-174</td>
<td>221</td>
</tr>
<tr>
<td>\Sigma y</td>
<td>13025</td>
<td>8492</td>
<td>11189</td>
</tr>
</tbody>
</table>
From this working table, we have

(1) \[ S_T = \sum y^2 - C = 44958 - O = 44958 \]

Notes:—"O" is the result of \((-2)^2 / 32\), the correction term, disregarding decimals less than 0.5. Fractions of 0.5 and over are counted as a whole number.

(2) From the \(\Sigma y\)-line of table 1. 2. 3 we have the following table 1. 2. 4.

<table>
<thead>
<tr>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>241</td>
</tr>
<tr>
<td>V2</td>
<td>-174</td>
</tr>
<tr>
<td></td>
<td>67</td>
</tr>
</tbody>
</table>

Using these (1) and (2), we have a logarithm of the potency we are looking for as follows:

\[
\log \theta_\beta = \frac{0.301(M2-M1)(V1-V2)}{n s_T} = \frac{-0.301 \times 136 \times 926}{32 \times 44958} = -0.02635 = 1.97365
\]

\[ \theta_\beta = 0.9411 \]

This is a value of potency of the sample M2 against the standard M1. 0.301 is the logarithm of 2, that is

\[
\text{dilution of } V2 = \frac{4000}{V1} = 2
\]

0.602 must be used when \( V1 = 1000 \) and \( V2 = 4000 \).

What does \( \theta_\beta \) mean? What is the relation of \( \theta_\beta \) to \( \theta_\alpha \)? \( \theta_\alpha \) is formulated as follows:

\[
\log \theta_\alpha = \frac{0.301(M2-M1)}{V1-V2}
\]

These questions will be answered in the next section. To get a potency from the data calculated according to the factorial designs which we will state later, we have

\[
\log \theta_\beta = \frac{0.301 \sqrt{s_M s_V}}{s_T}
\]

\[
\log \theta_\alpha = 0.301 \sqrt{\frac{s_M}{s_V}}
\]

How these formulae are obtained will be stated later. (§ 2.1 and § 2.3)

Moreover, the tests for significance of factors on which reliance is placed for judging the accuracy of the experiment will be discussed later. (§ 2.4) In this instance, the value 0.94 seems somewhat smaller than 1.0. But, testing the factor M, it turned out that \( s_M \) is non significant, that is to say that the difference between the test and the standard tuberculin is not sufficiently proved, so far as this experiment is concerned. Yet, we will proceed our calculation for the purpose of illustrating the computation method.

**REFERENCE**

CHAPTER 2. TWO-POINT-MEASUREMENT

§ 2.1 \( \theta_p \) AND \( \theta_e \). (continued from section 1.2)

Our estimation of a potency starts from the correlation between the dilution and the size of reaction. Of the example quoted in § 1.2, \( V_1 \) is 2000 times, \( V_2 \) 4000, the logarithms of which are 3.301 and 3.602 respectively. To simplify the calculation we deal with code numbers (x's) instead of original numbers (X's), where

\[
x = \frac{X - 3.301}{0.301}
\]

Accordingly we work with 0 and 1 instead of 3.301 and 3.602.

Arranging the items of the working table (table 1.2.3) in \( V_1 \) and \( V_2 \) respectively, we proceed in the following way to compute the correlation:

(See table 2.1.1)

| Table 2.1.1 Rearrangement of table 1.2.3 to compute the correlation. |
|------------------|-----------|-----------|
| \( x \)   | \( V_1 \) | \( V_2 \) |
| 0       | 56        | -14       |
| 68      | -30       |
| 8       | -38       |
| \vdots | \vdots    |
| 62      | -14       |
| 62      | -22       |
| 16      | -46       |
| \vdots | \vdots    |
| \( f \)  | 16        | 16        |
| \( \Sigma fx \) | 0        | 16        |
| \( \Sigma fx^2 \) | 0        | 16        |
| \( \Sigma y \)  | 462       | -464      |
| \( \Sigma xy \)  | 0         | -464      |

Note: \( f \) is the number of items corresponding to each column of \( x \).

From table 2.1.1 we have
\[
\bar{x} = \frac{\Sigma fx}{n} = 16/32 = 0.5
\]
\[
s_x^2 = \frac{\Sigma fx^2}{n} - \bar{x}^2 = 16/32 - 0.5^2 = 0.25
\]

Returning to the original unit \( X \) instead of working unit \( x \), we have
\[
s_X^2 = 0.301^2 \cdot s_x^2 = 0.301^2 \times 0.25
\]

Referring to (1) and (2) in (B) of section 1.2, we have
\[
\bar{y} = \frac{\Sigma y}{n} = (V_1 + V_2)/n,
\]
\[
s_y^2 = s_T/n
\]

Returning to the original unit \( Y \), we have
\[
s_Y^2 = s_Y^2/100^2 = s_T/100^2 n
\]
\[
p = (V_1 x + V_2 x_1)/n - \bar{x} \bar{y} = V_2/n - 0.5(V_1 + V_2)/n = (V_2 - V_1)/2n
\]

Returning to the original units of \( x \) and \( y \), we have
\[
P = (0.301)(V_2 - V_1)/(100 \times 2n)
\]
Let \( d = \frac{(M_2 - M_1)}{(n/2)} = 2\frac{(M_2 - M_1)}{n} \), and with original unit, we have
\[
D = \frac{2(M_2 - M_1)}{100n}.
\]
If we write \( \alpha \), we have
\[
\alpha = \frac{D}{\lvert \beta \rvert}.
\]
This is the formula we set out at the end of section 1.2.

To calculate the \( \log \beta \), we may use either
\[
\log \beta = \frac{2(M_2 - M_1)}{100n} \quad \text{or} \quad \log \beta = \frac{0.301(V_2 - V_1)}{V_1 - V_2}.
\]
The latter formula is simple in calculation. But the former is put up here to show what is \( \theta_\alpha \). We see that the \( \log \theta_\alpha \) is obtained by using the reciprocal of \( \alpha \). Calculating the \( \log \theta_\alpha \), we have
\[
\log \theta_\alpha = -0.04420 = 1.95580
\]
\[
\theta_\alpha = 0.9032
\]
\( \theta_\alpha \) denotes the potency of the tuberculin estimated by \( \alpha \). This \( \theta_\alpha \) is completely the same as the result calculated by Umezawa’s new method, the latter formula being compared with that given by him. It is even desirable to follow his formula in the computation of \( \theta_\alpha \), when the only \( \theta_\alpha \) is required to calculate in case of two-point-measurement, because this is a simpler way of calculation.

But, we prefer another value \( \theta_\beta \) to \( \theta_\alpha \). This \( \theta_\beta \) is implied in the following:
\[
\log \theta_\beta = d \times \left\lvert \beta \right\rvert = \frac{2(M_2 - M_1)}{100n} \times \frac{0.301 \times 100(V_1 - V_2)}{2 s_T}
\]
\[
= \frac{0.301(M_2 - M_1)(V_1 - V_2)}{n S_T}
\]
This is the formula we set out at the end of section 1.2.

To calculate the \( \log \theta_\beta \) we may use either \( D \times \left\lvert \beta \right\rvert \) or \( \frac{0.301(M_2 - M_1)(V_1 - V_2)}{n S_T} \). For the two-point-measurement, the latter formula is simple in calculation. The former, however, is applicable to any-point-measurement, 3-, 4-, 5- and 6-point and so on. Besides, in the course of calculation we are acquainted with other informations, i.e. \( \beta \) or \( r \), which will be of use in the discussion of the potency obtained.

We calculated the \( \theta_\beta \) in section 1.2, which was
\[
\theta_\beta = 0.9411.
\]

Now we have two values, \( \theta_\alpha \) and \( \theta_\beta \). Which is suitable for the estimation of potency? In this instance, the difference between 0.90 and 0.94 is out of question, as stated a bit at the end of section 1.2, because of being non-
significant of $S_M$ in the analysis of factorial design. In general it is not the case.

$\theta_\beta$ is right according to the theory of regression coefficient. To calculate unknown $x$ from known $y$ we must resort to the $\beta$, that is $p/s_y^2$. The $\alpha$, that is $p/s_x^2$, is used to obtain $y$ from $x$. It is wrong to utilise the reciprocal of $\alpha$ instead of $\beta$ for the estimation of $x$ from $y$.

Since $r^2 = \alpha\beta$, $1/\alpha$ is not equal to $\beta$ in general. When $r=1$, it becomes $1/\alpha = \beta$.

Umezawa advised the use of his method when the potency of test tuberculin lies between 0.5—1.5. This restriction may consequently bring forth a larger correlation coefficient. When the coefficient is about 0.8 or over, $1/\alpha$ may roughly be equal to $\beta$; but if it is some 0.5 or under, $1/\alpha$ is not equal to $\beta$. The potency calculated by $1/\alpha$ is exaggerated. When the test is stronger, the potency is expressed in too large a value, and when it weaker, the value is too small.

An example of too large a value is brought forward as follows. See table 2.1.2.

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V2</td>
</tr>
<tr>
<td>N1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>-107</td>
<td>-33</td>
</tr>
<tr>
<td>Q2</td>
<td>65</td>
<td>-12</td>
</tr>
<tr>
<td>N2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>-32</td>
<td>-45</td>
</tr>
<tr>
<td>Q2</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>N3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>7</td>
<td>-18</td>
</tr>
<tr>
<td>Q2</td>
<td>7</td>
<td>-45</td>
</tr>
<tr>
<td>N4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>-12</td>
<td>-38</td>
</tr>
<tr>
<td>Q2</td>
<td>1</td>
<td>-18</td>
</tr>
<tr>
<td></td>
<td>-40</td>
<td>-183</td>
</tr>
</tbody>
</table>

Notes:—

(1) Coded by $y=100Y-399$, measured on rednesses.
(2) M1........Standard, M2........Test.
(3) V1........500 times dilution, V2........2000 times.
(4) Q1........Fore half of the body, Q2........hind half.

Calculating similarly to the example shown above, we have

$\theta_\alpha = 3.813$
$\theta_\beta = 1.195$

The correlation coefficient is $-0.3649$, i.e. very small. The $\theta_\alpha$ is 3.8 compared with 1.2 of the $\theta_\beta$. The value of 3.8 is extravagant. In repeated experiments it was shown that the potency of M2 was in the neighbourhood of 1.2. Thus the value expressed by $\theta_\beta$ proved to be true.
\section*{§ 2.2 PROCESS OF ANALYSING THE FACTORS}

Let us calculate the factors from Table 1.2.3.

1. Calculation of $S_M$ which means squares of the factor $M$, i.e. the difference between the sample and the standard. We will copy here the same table as Table 1.2.4.

\begin{table}[h]
\centering
\begin{tabular}{ccc}
& M1 & M2 \\
V1 & 241 & 221 & 462 \\
V2 & -174 & -290 & -464 \\
\hline
\end{tabular}
\end{table}

\[ S_M = \frac{(67 - (-69))^2}{32} = 578 \]

2. Similarly, we have $S_V$ which means the difference according to dilutions of tuberculins.

\[ S_V = \frac{(462 - (-64))^2}{32} = 26796 \]

3. $S_{M\times V}$ is called interaction between $M$ and $V$. The meaning of interaction in the study of the potency problem will be discussed later.

\[ S_{M\times V} = \frac{(221 - 174) - (241 - 290))^2}{32} = 288 \]

4. To get $S_N$, i.e. differences according to individuality of the animals sensitized, we have the following Table 2.2.1 from the last column of Table 1.2.3.

\begin{table}[h]
\centering
\begin{tabular}{cccccc}
Q1 & N1 & N2 & N3 & N4 \\
& 90 & -60 & -75 & -15 & -60 \\
Q2 & 78 & -43 & -100 & 123 & 58 \\
\hline
168 & -103 & -175 & 108 & 58 \\
\end{tabular}
\end{table}

\[ S_N = \frac{168^2 + (-103)^2 + (-175)^2 + 108^2 + (-2)^2}{8} = 10140 \]

Here, "8" comes from the fact that each "N" is composed of eight items. $(-2)^2/32$ is counted as zero; this rounding was stated in section 1.2 where $S_T$ was calculated.

5. To calculate $S_Q$ which means the local difference between the fore and hind half of the body, we have

\[ S_Q = \frac{58 - (-60))^2}{32} = 435, \text{ using the last column of preceding table (Table 2.2.1).} \]

The result is the same as we calculate as follows:

\[ S_Q = \frac{58^2 + (-60)^2 + (-2)^2}{32} = 435. \]

This principle is common to the $S_M$ and $S_V$ stated above.

6. To calculate $S_{N\times Q}$ which means the interaction between $N$ and $Q$, we calculate $S_{NQ}$ at first. From Table 2.2.1 we have

\[ S_{NQ} = \]
Then we have
\[ S_{NQ} = \frac{90^2 + (-60)^2 + \ldots + (-100)^2 + 123^2}{4} - C = 12653 \]

(7) To calculate \( S_{M\times N} \) and \( S_{V\times N} \):

(a) Adding \( Q1 \) and \( Q2 \) together in each "N" on table 1.2.3, we have the following table 2.2.2.a.

<table>
<thead>
<tr>
<th></th>
<th>V1</th>
<th>V2</th>
<th>M2</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>124</td>
<td>-44</td>
<td>124</td>
<td>-36</td>
</tr>
<tr>
<td>N2</td>
<td>16</td>
<td>-52</td>
<td>17</td>
<td>-84</td>
</tr>
<tr>
<td>N3</td>
<td>17</td>
<td>-84</td>
<td>2</td>
<td>-110</td>
</tr>
<tr>
<td>N4</td>
<td>84</td>
<td>6</td>
<td>78</td>
<td>-60</td>
</tr>
</tbody>
</table>

This table is a step to proceed to table 2.2.2.b and table 2.2.2.c, and is used to calculate \( S_{M\times N} \) later.

(b) Adding \( V1 \) and \( V2 \) together in each "M", we have the following table 2.2.2.b. Calculating at first \( S_{MN} \), we have \( S_{M\times N} \) by subtracting \( S_M \) and \( S_N \) from \( S_{MN} \).

\[ S_{MN} = \frac{80^2 + (-36)^2 + \ldots + (-108)^2 + 18^2}{4} - C = 11127 \]

\[ S_{M\times N} = S_{MN} - S_M - S_N = 409 \]

(c) Adding \( V1 \) of \( M1 \) to \( V1 \) of \( M2 \), and \( V2 \) of \( M1 \) to \( V2 \) of \( M2 \) on table 2.2.2.a, we have the following table 2.2.2.c.

<table>
<thead>
<tr>
<th></th>
<th>V1</th>
<th>V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>248</td>
<td>-80</td>
</tr>
<tr>
<td>N2</td>
<td>33</td>
<td>-136</td>
</tr>
<tr>
<td>N3</td>
<td>19</td>
<td>-194</td>
</tr>
<tr>
<td>N4</td>
<td>162</td>
<td>-54</td>
</tr>
</tbody>
</table>

\[ S_{VN} = \frac{248^2 + 33^2 + \ldots + (-194)^2 + (-54)^2}{4} - C = 38662 \]

\[ S_{V\times N} = S_{VN} - S_V - S_N = 1726 \]

(8) To obtain \( S_{M\times V\times N} \), we calculate \( S_{MVN} \) at first from table 2.2.2.a.

\[ S_{MVN} = \frac{124^2 + (-44)^2 + \ldots + 78^2 + (-60)^2}{2} - C = 40257 \]

\[ S_{M\times V\times N} = S_{MVN} - S_M - S_V - S_N - S_{MVV} - S_{MVN} - S_{V\times N} = 320 \]
(9) To get $S_{MXQ}$ we sum up all "Q1" on every column, and all "Q2" respectively on table 1.2.3, and we have the following table 2.2.3.a.

Table 2.2.3.a

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>96</td>
<td>108</td>
</tr>
<tr>
<td>Q2</td>
<td>145</td>
<td>113</td>
</tr>
</tbody>
</table>

Table 2.2.3.a is used later to calculate $SMVQ$, and at first from this table we make the following table 2.2.3.b.

Table 2.2.3.b

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>-32</td>
<td>-23</td>
</tr>
<tr>
<td>Q2</td>
<td>99</td>
<td>-41</td>
</tr>
</tbody>
</table>

$SM_{MXQ} = \frac{(99 - 28 + 32 + 41)^2}{32} = 648$

Similarly we have table 2.2.3.c.

Table 2.2.3.c

<table>
<thead>
<tr>
<th></th>
<th>V1</th>
<th>V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>204</td>
<td>-264</td>
</tr>
<tr>
<td>Q2</td>
<td>258</td>
<td>-200</td>
</tr>
</tbody>
</table>

$SV_{XQ} = \frac{(204 - 200 - 258 + 264)^2}{32} = 3$

To get $S_{MXVXQ}$ we calculate at first $SMVQ$ from table 2.2.3.a, and then we get $SM_{MXVXQ}$ as follows:

$SMVQ = \frac{96^2 + 128^2 + \ldots + 113^2 + 154^2}{4} - C = 28847$

$SM_{MXVXQ} = SMVQ - S_M - S_V - S_Q - SM_{MXV} - SM_{QX} - SV_{XQ} = 99$

(10) To calculate $S_{MXNQ}$, we make the following table 2.2.4, adding V1 to V2 in each M, and we get at first $SM_{NQ}$, then $SM_{MXNQ}$.

Table 2.2.4

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 {</td>
<td>Q1</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Q2</td>
<td>38</td>
</tr>
<tr>
<td>N2 {</td>
<td>Q1</td>
<td>-30</td>
</tr>
<tr>
<td></td>
<td>Q2</td>
<td>-6</td>
</tr>
<tr>
<td>N3 {</td>
<td>Q1</td>
<td>-30</td>
</tr>
<tr>
<td></td>
<td>Q2</td>
<td>-37</td>
</tr>
<tr>
<td>N4 {</td>
<td>Q1</td>
<td>-14</td>
</tr>
<tr>
<td></td>
<td>Q2</td>
<td>104</td>
</tr>
</tbody>
</table>

$SM_{NQ} = \frac{42^2 + 38^2 + \ldots + (-1)^2 + 19^2}{2} - C = 14977$

$SM_{MXNQ} = SM_{NQ} - S_M - S_N - S_Q - SM_{MN} - SM_{XQ} - SV_{XQ} = 689$

(11) To calculate $SV_{XNQ}$, in the similar way to (10), we have table 2.2.5 and its calculations.
Table 2.2.5

\[
\begin{array}{c|c|c}
\text{N1} & \text{Q1} & 118 \\
   & \text{Q2} & 130 \\
\text{N2} & \text{Q1} & 24 \\
   & \text{Q2} & 9 \\
\text{N3} & \text{Q1} & 17 \\
   & \text{Q2} & 2 \\
\text{N4} & \text{Q1} & 45 \\
   & \text{Q2} & 117 \\
\end{array}
\]

\[
SV_{NQ} = \frac{118^2 + 130^2 + \ldots + (-60)^2 + 6^2}{2} = 41620
\]

\[
SV_{VXNQ} = SV_{NQ} - SV - SN - SQ - SV_{VXN} - SV_{XQ} - SN_{XQ} = 442
\]

(12) At last, we have

\[
SM_{VXNQ} = ST - SM - SV - SN - SQ - SM_V - SM_Q - SM_{VQ} - SM_{QV} - SM_{NVXQ} - SM_{VXQ} = 307
\]

Thus, we have finished all the calculations of factors. They are tabulated as follows (Table 2.2.6)

Table 2.2.6

<table>
<thead>
<tr>
<th>Factors</th>
<th>f</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>1</td>
<td>578</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>26796</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>10140</td>
</tr>
<tr>
<td>Q</td>
<td>1</td>
<td>435</td>
</tr>
<tr>
<td>M\times V</td>
<td>1</td>
<td>288</td>
</tr>
<tr>
<td>M\times N</td>
<td>3</td>
<td>409</td>
</tr>
<tr>
<td>M\times Q</td>
<td>1</td>
<td>648</td>
</tr>
<tr>
<td>V\times N</td>
<td>3</td>
<td>1726</td>
</tr>
<tr>
<td>V\times Q</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>N\times Q</td>
<td>3</td>
<td>2078</td>
</tr>
<tr>
<td>M\times V\times N</td>
<td>3</td>
<td>320</td>
</tr>
<tr>
<td>M\times V\times Q</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>M\times N\times Q</td>
<td>3</td>
<td>689</td>
</tr>
<tr>
<td>V\times N\times Q</td>
<td>3</td>
<td>442</td>
</tr>
<tr>
<td>M\times V\times N\times Q</td>
<td>3</td>
<td>307</td>
</tr>
<tr>
<td>T</td>
<td>31</td>
<td>44958</td>
</tr>
</tbody>
</table>

SS means sum of squares, and is indicative of how far the factor is concerned in this experiment.

"f" means degree of freedom. What does the degree of freedom mean? It is very difficult to state in short. The reader is advised to refer to books of statistics. As far as this example is concerned, it is convenient to learn that the degree of freedom is

Suffix number—1.

M has two sorts of suffix, M1 and M2; the same for V and Q. So, M has 2−1=1 for the degree of freedom; the same for V and Q. N has four suffix numbers, N1, N2, N3 and N4. N has, therefore, 4−1=3 degrees of freedom. For M\times V, we have (2−1)(2−1)=1. For M\times N, (2−1)(4−1)=3. For M\times V\times N, we have (2−1)(2−1)(4−1)=3. For M\times V\times Q (2−1)(2−1)(2−1)=1.
We have dealt with four principal factors, M, V, N and Q. In addition, we have R and H in further studies. R denotes reaction, i.e., induration and redness. H means 24 hours' reading and 48 hours'. Computing these six factors in one table, we have a more troublesome task ahead.

What is the use of such a troublesome task? It will make itself clear in later sections.

§ 2.3 POTENCY AND CORRELATION ETC. OBTAINED FROM THE ANALYSES OF FACTORIAL DESIGN

Once the factorial design is analysed, it will be convenient to get the potency, the regression coefficient and correlation coefficient by using the figures of analyses.

Since \( \frac{(M_2-M_1)^2}{n} = S_M \), and \( \frac{(V_1-V_2)^2}{n} = S_V \),

then \( \log_\theta = \frac{0.301(M_2-M_1)(V_1-V_2)}{n \cdot S_T} = 0.301 \frac{\sqrt{S_M \cdot S_V}}{S_T} \)

and \( \log_\alpha = \frac{0.301(M_2-M_1)}{V_1-V_2} = 0.301 \sqrt{\frac{S_M}{S_V}} \).

These are the formulae we stated at the end of section 1.2.

As for the correlation between dilutions and reactions, we have as follows:

Since \( p = \frac{V_2-V_1}{2n} = \frac{1}{2} \sqrt{\frac{S_V}{n}} \)

and \( s_x^2 = 0.25 \), a constant in our case, where \( x \) is classified in two ranks,

then \( \alpha = \frac{p}{s_x^2} = \frac{1}{2} \sqrt{\frac{S_V}{n}} \cdot 0.25 = \frac{1}{2} \sqrt{\frac{S_V}{n}} \).

Since \( s_y^2 = \frac{S_T}{n} \), then \( \beta = \frac{p}{s_y^2} = \frac{1}{2} \sqrt{\frac{S_V}{n}} \cdot \frac{S_T}{n} = \sqrt{\frac{nS_V}{2S_T}} \).

These \( \alpha \) and \( \beta \) are expressed in their working units, not in original units.

As for the correlation coefficient, we have

\[ r^2 = \alpha \beta = \frac{S_V}{n} \cdot \sqrt{\frac{nS_V}{2S_T}} = \frac{S_V}{S_T} \]

\[ r = \sqrt{\frac{S_V}{S_T}} \]

\( \beta \) and \( r \) will often be seen in later discussions.

It must be noted that such simple relations as stated above do not hold good for 3- or more-point-measurement.

§ 2.4 HOW TO TEST THE SIGNIFICANCE OF A FACTOR

In the example of section 1.2 we obtained a value 0.94 for the potency of a tuberculin. This 0.94 seems somewhat smaller compared with the unity 1.0. But, it is imprudent to say at once, “weak.”
First of all, we test the significance of the factor M. To do this, the question is how to find the error variance, or the sum of squares due to error divided by its degree of freedom.

When the factorial design is all calculated, we may pick up the squares appropriate for our purpose. In this example, we pick up the factors concerned with M as follows, (table 2.4.1)

Table 2.4.1 Picking up of factors to find the error variance.

<table>
<thead>
<tr>
<th>Factors</th>
<th>SS</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>M×V</td>
<td>288</td>
<td>1</td>
</tr>
<tr>
<td>M×N</td>
<td>400</td>
<td>3</td>
</tr>
<tr>
<td>M×Q</td>
<td>648</td>
<td>1</td>
</tr>
<tr>
<td>M×V×N</td>
<td>320</td>
<td>3</td>
</tr>
<tr>
<td>M×V×Q</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>M×N×Q</td>
<td>689</td>
<td>3</td>
</tr>
<tr>
<td>M×V×N×Q</td>
<td>306</td>
<td>3</td>
</tr>
</tbody>
</table>

2759/15 is the error variance for testing the factor M, and we have

\[ F_0 = \frac{2759}{15} = 3.14 \]

This 3.14 is smaller than 4.54 which is a 5% point on Snedecor's F-distribution. The factor M, therefore, is non-significant, that is to say that the difference between the test and standard is not significantly proved, so far as this experiment is concerned.

When only the factor M is required to test its significance, we may dispense with the full calculation of the factorial design, and proceed as follows:

Following the same way as stated in section 2.2 (1), we have

\[ S_M = 578, \quad f = 1, \]

and the same way as in section 2.2 (11), we have

\[ S_{VQ} = 41620, \quad f = 15. \]

Here, \( f = (\text{Numbers of squares made}) - 1 \)

\[ S_M + S_{VQ} = 42198, \quad f = 16 \]

\[ S_T = 44958, \quad f = 31 \]

2760 is the error variance for testing the factor M, which must be the same as the one we obtained by pick-up-method. The discrepancy between 2759 and 2760 is of great importance, coming from rounding errors.

(2) Significance of the factor V.

Similarly to the case of M, we have as to V:

(a) by picking up

<table>
<thead>
<tr>
<th>Factors</th>
<th>SS</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>M×V</td>
<td>288</td>
<td>1</td>
</tr>
<tr>
<td>V×N</td>
<td>1726</td>
<td>3</td>
</tr>
<tr>
<td>V×Q</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>M×V×N</td>
<td>320</td>
<td>3</td>
</tr>
<tr>
<td>M×V×Q</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>V×N×Q</td>
<td>442</td>
<td>3</td>
</tr>
<tr>
<td>M×V×N×Q</td>
<td>307</td>
<td>3</td>
</tr>
</tbody>
</table>

3185 is the error variance for testing the factor V.
(b) by saving the full calculation

\[ S_Y = 26796, \quad f = 1 \]
\[ S_{MNQ} = 14977, \quad f = 15 \]

\[ \frac{41773}{3185} = 16 \]
\[ S_T = 44958, \quad f = 31 \]

\[ \frac{3185}{15} = 205 \] (as before)

And variance ratio of Snedecor is

\[ \frac{26796}{3185/15} = 126 \]

Such a large value as 126 is needless to refer to the F-distribution table. Of course, \( S_Y \), i.e. the difference between 2000 and 4000 times dilutions is significant.

(3) Significance of the factor \( S_{M \times V} \).

(a) by picking up

<table>
<thead>
<tr>
<th>Factor</th>
<th>SS</th>
<th>( f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( M \times V \times N )</td>
<td>320</td>
<td>3</td>
</tr>
<tr>
<td>( M \times V \times Q )</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>( M \times V \times N \times Q )</td>
<td>307</td>
<td>3</td>
</tr>
</tbody>
</table>

\[ \frac{726}{7} = 103.7 \]

(b) without full calculation of factors

\[ S_{M \times V} = 288, \quad f = 1 \]
\[ S_{YNQ} = 41620, \quad f = 15 \]
\[ S_{MNQ} = 14977, \quad f = 15 \]

\[ 56885 \]
\[ 31 \]
\[ S_T = 44958, \quad f = 31 \]
\[ S_{NQ} = 12653, \quad f = 7 \]

\[ \frac{57611}{38} = 1511 \]
\[ \frac{56885}{31} = 1835 \]

\[ \frac{726}{7} = 103.7 \] (as before)

\[ F_0 = \frac{288/1}{726/7} = 2.77 \]

2.77 is smaller than 5.59 referred to on the F-distribution table, showing that the interaction between \( M \) and \( V \) is non-significant.

§ 2.5 CONFIDENCE LIMITS OF THE POTENCY CALCULATED BY OUR FORMULA.

The measurement of potency by animal experiments is subject to many factors or errors, the analysis of which is given in the factorial design. In view of this analysis we aim at the confidence limits of the potency calculated
TUBERCULIN

by our formula. Although we know far too little as yet about the mathematics of this matter, we have two ways to cope with this problem. The one is the method of section 1.1. (a), the other is a method using the analysis of factorial design.

(1) By the method of section 1.1. (a) we are able to know the fiducial limits of the estimate of potency, and to test the difference, if any, between a value estimated by induration and the other estimated by redness. Similarly we test the difference between a potency estimated at 24 hours and at 48 hours. We omitted the method of section 1.1. (a) for two-point-measurement. But the principle is the same.

(2) The method using the analysis of factorial design:—

(a) Testing of the factor M is useful. If SM falls short of significance, it is plain that the potency of the test tuberculin cannot be distinguishable from the standard.

(b) Using the F-distribution table inversely, we will find the range of errors within which SM falls short of significance. (See later, section 3.4)

(c) We expect always that Sv is significant. If Sv falls short of significance, we should say that the experiment is too erroneous to be relied upon.

(d) The same can be said about the test of r and $\beta$.

\[ r = \sqrt{\frac{S_v}{S_T}} \quad \text{and} \quad \beta = \sqrt{\frac{nS_v}{2S_T}} \]

(e) If $S_{R\times M}$ is significant, we are led to think that there may be some difference in potency between the one estimated by induration and other by redness. But, as the potency is much influenced by $\beta$, we must be very careful in deciding this matter. The same holds for $S_{H\times M}$ the difference between 24 hours’ and 48 hours’ reading.

(f) In the case in which $S_{R\times M}$ proved significant and yet the potency on induration does not seem very different from the potency on redness, it is advisable to make a successive experiment. For example, we have here an M2 which is weaker than M1, $S_{R\times M}$ proved significant and yet the potency on induration, though large, does not seem very different from redness. We must design a successive experiment in such a way that the potency of M2 measured on induration is equivalent or somewhat stronger than M1 which is diluted properly to match M2. If the potency on redness shows a significantly smaller value, we shall be able to say that there is a difference between the potency measured on induration and the one measured on redness.

Without this successive experiment the first method (the calculation described in section 1.1. (a)) may prove at once if there is any difference.

(g) The method using the analysis of factorial design may have to be elaborated still further. The potency is implied in the equation

\[ \log \theta = 0.301 \sqrt{\frac{S_m \cdot S_v}{S_T}} \]

or

\[ \log \theta = D \times |\beta|. \]
Now we are able to test $S_M$ or $S_V$ separately. But how can we test $1/S_M S_V/S_T$ as a whole? We are able to test $D$ and $\beta$ separately. But how can we test $D \times |\beta|$ as a whole?

We are successful to test $\log \beta$ as a whole by the method described in1.1. (a), calculating elemental potencies on each NQ. I am afraid you might be very disappointed to find how wide the fiducial limit is extended. In another words, the inaccuracy of the experiment is very discouraging.

In this test the errors due to different sensitivity among guinea pigs (N) and different reaction among locations in the same individual (Q) are taken into account. And the elemental potency may variate in a wide range. On the contrary we are able to exclude the influence of NQ by analysing the factors, thus making the test more accurate. But, to my regret, the test cannot be applicable to the potency as a whole, as stated above.

In later sections we will deal with this problem illustrating many examples.

§ 2.6 IS THERE ANY DIFFERENCE IN SIZE BETWEEN REACTIONS ELICITED BY 0.1 ml OF 1000 TIMES DILUTED TUBERCULIN AND OTHERS ELICITED BY 0.2 ml OF 2000 TIMES DILUTION?

Example:—M1...........0.1 ml of 1000 times dilution, 
M2...........0.2 ml of 2000 times dilution.

The data are put up in table 2.6.1

<table>
<thead>
<tr>
<th>Ind.</th>
<th>Red.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 M1</td>
<td>6 × 7 6 × 5</td>
</tr>
<tr>
<td>M2 M1</td>
<td>6 × 6 4 × 4</td>
</tr>
<tr>
<td>N2 M1</td>
<td>6 × 8 7 × 9</td>
</tr>
<tr>
<td>M2 M1</td>
<td>6 × 7 8 × 7</td>
</tr>
<tr>
<td>N3 M1</td>
<td>11×11 12×12</td>
</tr>
<tr>
<td>M2 M1</td>
<td>10×9 11×11</td>
</tr>
<tr>
<td>N4 M1</td>
<td>7 × 7 7 × 6</td>
</tr>
<tr>
<td>M2 M1</td>
<td>7 × 7 6 × 6</td>
</tr>
</tbody>
</table>
Arranging the data on induration, we have the following table 2.6.2.

Table 2.6.2

<table>
<thead>
<tr>
<th>N</th>
<th>Q</th>
<th>M1</th>
<th>M2</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>6.0</td>
<td>6.5</td>
<td>-20</td>
<td>-15</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>8.0</td>
<td>6.5</td>
<td>0</td>
<td>-15</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>7.0</td>
<td>7.0</td>
<td>-10</td>
<td>-10</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8.5</td>
<td>6.5</td>
<td>5</td>
<td>-10</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>7.0</td>
<td>6.5</td>
<td>-10</td>
<td>-15</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>7.5</td>
<td>7.5</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>8.0</td>
<td>7.5</td>
<td>0</td>
<td>-5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>9.0</td>
<td>9.0</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
\[ y = 10Y - 80 \]

\[ C = 80^2 / 32 = 200 \]

\[ S_T = 5850 - 200 = 5650 \]

\[ S_M = 10^2 / 32 = 3, \quad f = 1 \]

\[ S_{NQ} = 10550 / 2 - 200 = 5075, \quad f = 15 \]

\[ S_M = \]

\[ \frac{5078}{16} \]

\[ S_T = \]

\[ \frac{5650}{31} \]

\[ \frac{572}{15} \]

\[ F_o = \frac{3/1}{572/15} = \frac{45}{38} < 1 \]

\[ S_M \text{ is non-significant.} \]

On redness, we have the following table 2.6.3 and its calculation:

Table 2.6.3

<table>
<thead>
<tr>
<th>N</th>
<th>Q</th>
<th>M1</th>
<th>M2</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>4.0</td>
<td>5.5</td>
<td>-40</td>
<td>-25</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>6.0</td>
<td>5.0</td>
<td>-20</td>
<td>-30</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>5.0</td>
<td>5.0</td>
<td>-30</td>
<td>-30</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8.5</td>
<td>6.0</td>
<td>5</td>
<td>-20</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>8.0</td>
<td>7.5</td>
<td>0</td>
<td>-5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>8.0</td>
<td>7.5</td>
<td>0</td>
<td>-5</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>8.0</td>
<td>6.5</td>
<td>0</td>
<td>-15</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>11.0</td>
<td>9.5</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>

\[ y = 10Y - 80 \]
In the example, we see that $S_M$ is non-significant. This shows that the distinction between the reaction elicited by 0.1 ml of 1000 times diluted tuberculin and the reaction by 0.2 ml of 2000 times dilution cannot significantly be proved by these small samples, which may usually be used to obtain a rough estimate of potency.

Such being the case, the injection method of this kind is used in the most examples quoted in the next section (§ 2.7). Of course, it is advisable to make two sorts of dilutions even in the case of two-point-measurement, injecting the same quantity of 0.1 ml of $V_1$ and $V_2$ respectively.

§ 2.7 INTERPRETATION OF INTERACTION BETWEEN VARIOUS FACTORS.

In the problem of potency test we bear in mind the following six factors:—

1. H. Reading at 24 hours and 48 hours.
2. R. Measurement on induration or on redness.
4. V. Degrees of dilutions.
5. N. Difference of sentitivity according to the individuality of the test animals.
6. Q. Difference of the reaction according to the site of injection.
It is very laborious to calculate all these factors and their combinations. We do not make such cumbersome calculations as a routine work. Because, in a common test under good conditions, in which the sensitivity of animals is moderate and the technique of injection is satisfactory, we have no difficulty in attaining a judgment without hesitation. At most a test of the significance of $S_M$ should be made. Because, some degree of discrepancy between the two specimen may be of no significance in small samples of the experiment.

The following study of interactions of several factors is rather of theoretical one than of practical. But, it is suggestive of how the result, be it adequate or not, was derived from.

<table>
<thead>
<tr>
<th>Factors</th>
<th>L1</th>
<th>L3</th>
<th>L6</th>
<th>L7</th>
<th>L(7)</th>
<th>L(6)</th>
<th>NaCl</th>
<th>Auto</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td></td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>_</td>
<td>_</td>
<td>***</td>
</tr>
<tr>
<td>R</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
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<td>_</td>
<td>***</td>
</tr>
<tr>
<td>M</td>
<td>***</td>
<td></td>
<td>***</td>
<td>*</td>
<td>_</td>
<td>_</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>V</td>
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<td></td>
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<td></td>
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<tr>
<td>N</td>
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<td>**</td>
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<td></td>
</tr>
<tr>
<td>Q</td>
<td></td>
<td>**</td>
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<td>**</td>
<td>_</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H×R</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
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<td>***</td>
<td>***</td>
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<tr>
<td>H×M</td>
<td>**</td>
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<td>_</td>
<td></td>
</tr>
<tr>
<td>H×V</td>
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<td>**</td>
<td>***</td>
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<td>_</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>H×N</td>
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<td>***</td>
<td>***</td>
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<td>***</td>
<td>***</td>
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<tr>
<td>H×Q</td>
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<td></td>
</tr>
<tr>
<td>R×M</td>
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<td>_</td>
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<tr>
<td>R×V</td>
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<tr>
<td>R×N</td>
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</tr>
<tr>
<td>R×Q</td>
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<tr>
<td>M×V</td>
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<td>_</td>
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<tr>
<td>M×N</td>
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<td>*</td>
<td>***</td>
<td>_</td>
<td>**</td>
<td>_</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M×Q</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V×N</td>
<td>*</td>
<td>_</td>
<td>_</td>
<td>***</td>
<td>_</td>
<td>_</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V×Q</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N×Q</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>_</td>
<td>**</td>
<td>*</td>
<td>_</td>
<td></td>
</tr>
</tbody>
</table>

See table 2.7. L1, L2, L3 etc. are the samples tested. The blank rows show whether not yet calculated or needless to calculate. For instances, H is always significant; V is significant also; N is also significant if the sensitivity of animals are not the same. * means significant at 5% point of F-distribution, ** 1% point, *** 0.1% point or under. – means non-significant.

H×R is always significant, and this shows that the redness, being larger than the induration at 24-hour-reading, fades rapidly away and becomes smaller than the induration at 48-hour-reading.

H×N is always significant, and this shows that the individuality of test animals is not constant in the interval from 24 hours to 48 hours.

That R×V is significant shows that $(V1-V2)$ measured by induration differs from $(V1-V2)$ measured by redness.
If $R \times M$ be significant, we suspect that the sample is superior, say, to the standard on the capacity of producing induration, but the former is inferior to the latter in respect to produce redness. Such examples are in detail discussed in sections 3.1, 3.2 and 6.1.

That $R \times N$ is significant shows that an individuality in respect to induration does not go on the parallel way with the individuality in respect to redness. This is the case in most if not all experiments.

That $M \times N$ is significant shows that the individuality of the test animal to the standard tuberculin is not common to that of the sample. Thus an unaccordance of potency may sometimes be suspected, if the sensitivity of the test animals is very different in two experiments. But, this problem must be considered simultaneously with $V \times N$.

If $M \times V$ is significant in the case where $V_1 - V_2$ is larger in the sample than in the standard, we suspect that the sample becomes significantly weaker by dilution.

There are many other interactions to deal with, for instances $M \times Q$, $H \times Q$ etc.; but we will not discuss these any more, as the interactions not combining with $M$ or $V$ seem to have little relationship to the potency problem.

We will also omit any comment about the interactions of three or more factors. They are not completely worked out yet.

§ 2.8 LOCAL DIFFERENCES AMONG CUTI-REACTIONS.

Cutii-reaction varies in size and intensity due to location. Behind the nape of the neck the reactions are very variable, sometimes small sometimes large. The lumber region toward the rump of the animal gives a large reaction in most cases. In two-point-measurement, therefore, where four pairs of injections are made from the neck to the rump, the reactions in the hind part of the body are larger than those in the fore part. This is shown in table 2.7. We see that $S_Q$ is significant in the majority of cases.

In the three-point-measurement the injections are made in the middle part of the body, to avoid any variance due to location as far as possible. We have seldom met with cases where $S_Q$ showed significant results. This $Q$ has, nevertheless, another meaning, of which we noted in section 1.1(5). In the many-point-measurement, local differences are inevitable. To counterbalance this we have no means but to change the location one by one on animals chosen for the experiment to be of almost equal sensitivity by preliminary injections of a tuberculin.